Continuous metabolic monitoring techniques

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Chapter 5

Influence of implantation methods on performance of thin-film lactate and glucose micro-biosensors

Abstract

Introduction: Subcutaneously implanted lactate and glucose sensors may assist in optimising treatment in intensive care and of diabetic patients through automated, reliable and close monitoring of blood levels. Sensors are commonly implanted in subcutaneous adipose tissue, but this site yields inconsistent results.
Methods: To explore alternative implantation sites, twenty four thin-film amperometric lactate-glucose sensors were inserted in adipose tissue, striated muscle, or loose connective tissue of pigs. Sensor baselines and response times were compared, as recorded during an intravenous lactate/glucose injection.
Results: Baselines and responses were found generally close to zero in adipose and muscle tissue. In connective tissue, average lactate and glucose baseline levels ± SD were 80.5±19.1% and 90.9±15.7% of blood; and the 95% response times were 5.5±2.1 and 17.1±8.5 minutes respectively. The use of an intravenous injection of the sensor's analyte was found to be a quick way to check whether a newly implanted sensor is in close kinetic contact with blood.
Conclusions: The results indicate that subcutaneous loose connective tissue may be a better site for sensor implantation than the commonly used adipose tissue.

Introduction

A threatened energy supply to the brain or heart is an acute risk for tissue damage and eventually death because energy reserves in these tissues lasts only minutes. Such problems can occur e.g. in diabetic and intensive care patients through disturbed oxygen or glucose transport. Timely treatment of sudden ischemia or hypoglycaemia has been shown to decrease complications and death. A major problem is that many patients do not feel the impending danger, e.g. one-third of heart attack victims feels no chest pain[1], and many diabetic patients have little hypoglycaemia awareness. Tissue hypoxia is, however, indicated early in many patients by a lactate concentration increase, and has been documented to be a strong prognostic factor for complications and survival[2]. Frequent serial lactate or glucose measurement is thusfar sparsely practised, because of the heavy burden of frequent blood sampling and analysis for patients and physicians. Next, the data are not continuous, and often not instantly available for making therapeutic decisions. For further improvement of treatment of critically ill and diabetic patients, continuous subcutaneous lactate and glucose sensors combined with microdialysis have been proposed as an alternative. Automated, continuous, timely blood concentration data are advantageous over current off-line monitoring techniques. Placement of a probe subcutaneously is preferred, as such approach does not carry the risks of intravascular sensors. The first demands before clinical application of such subcutaneous sensor are of course the reliable and timely following of blood levels.

Current lactate and glucose sensors perform very accurate and with short response times in vitro[3-5]. Subcutaneously, however, a divergence from blood concentrations has been observed, e.g. with microdialysis[6;7], with ultrafiltration[8;9], and with directly implanted sensors[5;10;11]. This variable divergence is repeatedly seen, though very diverse techniques were employed for subcutaneous measurements. The reasons for this in vivo divergence have not been elucidated completely. Some of the previous results may be explained by inhibition of glucose oxidase activity by serum components, diffusional barrier formation by protein adsorption, and hydrogen peroxide consumption by granulocytes[12]. These factors, however, do not appear to inhibit thin-film sensors, which have proven to function stable and reliable in a continuous, heparinised blood stream[5]. Nevertheless, thin-film sensors combined with open-flow microperfusion suffer from the same divergence of subcutaneous concentrations from blood[13]. Possible mechanisms affecting the measurements in vivo may be related to tissue physiology or pathophysiology near the

implanted sensor. Candidate mechanisms are the damage to cells and vessels on the one hand, and local glucose to lactate metabolism in tissue on the other hand. These may impair the exchange of glucose or lactate between tissue near the sensor and the blood circulation through a barrier around the implantation wound and an increased diffusion distance to the nearest patent capillaries. The variation in structure of different tissues and the damage at implantation may explain the variable and low sensor responses, but has not yet been paid much attention.

We tested in pigs three potential subcutaneous sensor locations, to search a compartment that is in close kinetic contact with blood, because this information can be useful in later clinical investigations. Continuous, fast responding thin-film sensors[5] were placed subcutaneously in adipose, in striated muscle, and in loose connective tissue. These three tissues were chosen because they are all clinical applicable, but differ in metabolic rate and interstitial structure. The tissues' baseline was compared to the blood level, and the response time in vivo was determined for each individual sensor with an intravenous glucose/lactate injection, in an attempt to check in a quick way the sensor function. Simultaneous lactate and glucose measurements could be compared for interdependency, because the thin-film device combines both sensors next to each other.

Methods

Thin-film sensor

The characteristics of the thin-film lactate-glucose sensors employed have extensively been described previously[4;5]. These sensors consist of electrodes on the tip of a flexible polyimide carrier strip (width: 0.7mm) The distance between lactate and glucose working electrodes is 2 mm. The coated layers with either glucose or lactate oxidising enzymes produce a linear amperometric response range up to 40 mM (glucose) or 20 mM (lactate) in combination with in vitro and in serum 90% response time below 30 seconds.

Mass transfer of glucose and lactate is limited by the sensor membranes, averting exhaustion of the substrate in the sample contact layer. Possible in vivo interferents of electrodes as ascorbic acid, uric acid, and paracetamol have been found negligible. The correlation coefficient in heparinised blood was r=0.98 for glucose, and r=0.93 to r=0.99 for lactate.

Experimental procedures

Experiments were performed on four Yorkshire swines weighing 50, 38, 35, and 35 kg, each with six lactate-glucose sensors. The animals were maintained on a normal diet. All procedures were performed under anaesthesia with a combination of ketamine (15 mg/kg;), acepromazine (0.2 mg/kg) and atropine (0.05 mg/kg) given intramuscularly. An endotracheal tube was inserted for O_2/NO_2 ventilation. Ventilation with 2 % isoflurane (Forene®, Abbott, U.S.A.) was used to ensure adequate anaesthesia throughout the experiments. Levodromaron (2mg subcutaneously) was given as analgesic at the beginning of the procedure and supplemented if necessary. Lidocaine (50mg intravenously) was given prophylactically because of an experiment, separate from the present, which was performed subsequently on the same animal. Throughout the experiment, the condition of the animals was carefully monitored by means of continuous transcutaneous oximetry, intra-arterial blood pressure measurement, and electrocardiographic registration (ECG) of the limb leads and one modified precordial lead. The total time of sensors in vivo was 1.5-2 hours. To measure the response time in vivo, 0.25g/kg of glucose 40% and 0.1 g/kg of lactate 20% solution in 0.9% saline solution was injected intravenously in 2 minutes time after allowing the sensors to settle in for 45 minutes. Whole blood samples (2 ml) were withdrawn every 2, 5, or 10 minutes from a catheter in the coronary sinus vein. No heparin was used in this experiment to avoid any possible interference of anticoagulation on the measurements in tissue. The test tubes contained sodium fluoride and potassium oxalate to arrest coagulation and metabolism. The tubes were kept on ice until analysis. Concentrations of glucose and lactate were measured in whole blood and blood plasma after centrifugation with the Vitros 750 analyser (manufacturer: Ortho-Clinical Diagnostics, Illkirch Cedex, France) in the hospital routine laboratory. In order to make correct comparisons with sensor determinations, we adjusted the whole blood glucose concentrations with +15% to the molality in whole blood (in accordance with the manufacturer's report). Glucose molarity (mol/l blood) of whole blood is different from the molality (mol/kg water) as only about 80% of the blood volume consists of water[14]. The thin-film sensor measures the activity of glucose in water, hence the molality. Lactate was determined in blood plasma.

Description of the different insertion techniques

A total of twenty four lactate-glucose sensors were placed with a 1.25mm o.d. needle put through a lifted tissue fold. Each sensor was inserted from the open end into middle of the needle, then the needle was withdrawn gently, leaving

the sensor behind. In half of the experiments, 0.1ml of sterile saline was injected slowly while retracting the needle. Figure 1 shows an anatomic scheme of the different placements of the sensor. The sensors were placed either in abdominal adipose tissue (n=8), or intramuscularly in the abdominal wall (n=4), or in dermal loose connective tissue on the inside of the upper hind leg (n=12) (The latter sensors can be seen on the picture in figure 2). The sensor strips were fixated externally with tape on the skin.
Sensor electric currents in buffer with or without standards of glucose and lactate were measured before and after the experiments. The in vivo measured currents were converted to concentrations by first subtracting the baseline current in buffer from all measurements. Subsequent, the vivo measurements were calibrated with the standard curve determined before the experiment.
All procedures were reviewed and approved by the Animal Experiments Committee of the Groningen University.

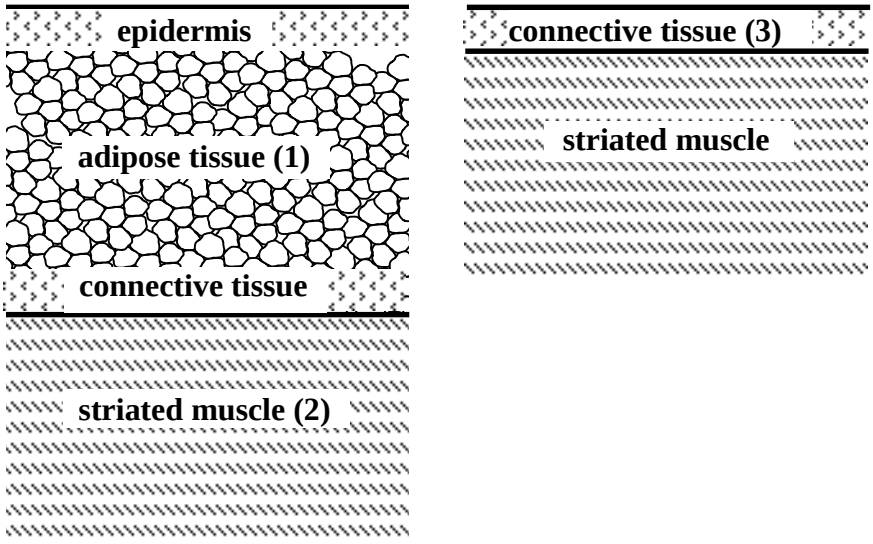

Figure 1
Scheme of anatomic layers of the abdominal wall (left) and the inside of the upper hind leg (right) in section. Sensors were inserted in adipose tissue (1), striated muscle tissue (2), and dermal loose connective tissue (3).

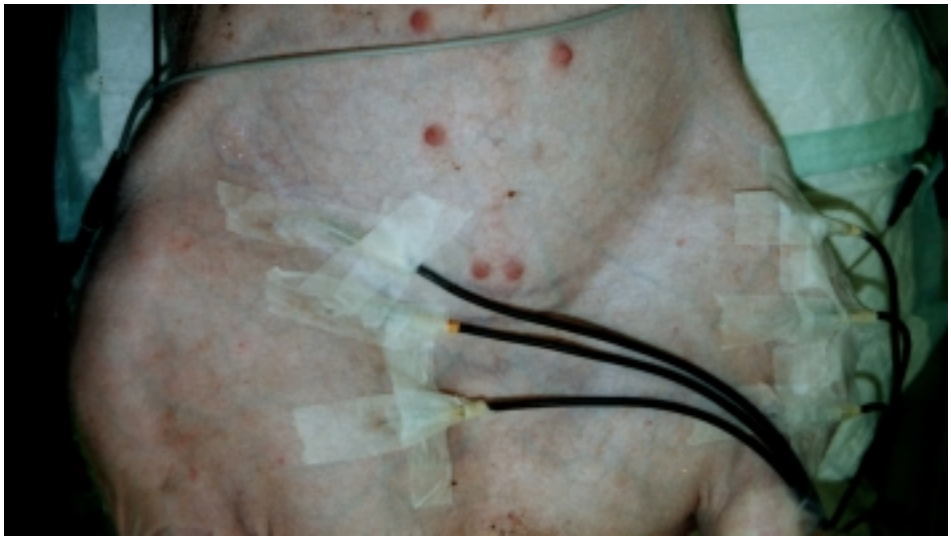

Figure 2
Six sensors placed in subcutaneous loose connective tissue on the inside of the upper hind leg.

Data presentation and statistics
The sensor data in figure three have been adjusted for presentation in graphics by presenting the datapoints as the average of nine datapoints collected in one minute in order to eliminate short wave electrical noise.
Baseline and response time results are presented as averages ± standard deviation (SD).

Results

The sensitivity of the sensors was 3.50 ± 1.84 nA/mM in vitro. The in vivo results of the experiments are summarised in table 1. Sensor baseline and response were generally close to zero in striated muscle and adipose tissue. Injection of saline together with a sensor in loose connective tissue removed the response to intravenous lactate-glucose injections.
In loose connective tissue, average sensor baselines ± SD were 80.5±19.1% and 90.9±15.7% of blood lactate and glucose levels respectively. The 95% response time was here 5.5±2.1 and 17.1±8.5 minutes. Examples of sensor responses to intravenous bolus injections are shown in figure 3. Lactate and glucose response

Table 1
Glucose and lactate baseline measurements intravenously (i.v.), and in three different tissues with sensors placed with or without injection of saline. The 95% response time to the i.v. glucose-lactate injection is also given (- indicates no response).

Tissue type	**Adipose tissue**		**Striated muscle**	**Connective tissue**	
saline injected(ml)	0	0.1	0 {0.1}	0	0.1
number of sensors	4	4	3 {1}	6	6
glucose i.v. baseline (mM)	4.15	6.70	4.15 {6.70}	3.94	3.29
sensor baseline ±SD (mM)	1.15,0,0,0	9.12,8.58,0,0	2.12,0,0 {8.40}	3.58±0.62	3.54±2.11
95% response ±SD (min.)	-,-,-,-	8,10,-,-	-,-,- {8}	17.1±8.5	-,-,-,-,-,-
lactate i.v. baseline (mM)	1.3	1.3	1.3	2.2	1.2
sensor baseline ±SD (mM)	5.48,0,0,0	4.51,4.10,0,0	1.38,0,0 {0}	1.77±0.42	1.25±1.06
95% response ±SD (min.)	-,-,-,-	10,-,-,-	-,-,- {-}	5.5±2.1	10,7,-,-,-,-

curves l.1 and g.1 are typical examples of sensors placed in adipose tissue. The fastest (sensors l.2 and g.2) and the slowest (sensors l.3 and g.3) response are shown of the sensors in connective tissue (sensors placed without saline injection).
The average intravenous levels of lactate and glucose are plotted versus connective tissue levels in figure 4. The labels near the graph indicate the sequence in time (in minutes) after the intravenous bolus injection at t = 0 minutes. The line of equality illustrates the (time-dependent) differences between both measurements. The graph indicates that interstitial glucose at baseline (-1 minute) and beyond 18 minutes after i.v. injection are at apparent steady state and near bloodplasma levels of glucose. Similarly, steady state lactate levels were close at -1 minute in the blood and interstitial compartments, but after the intravenous bolus injection, the 2 compartments equilibrated on a significantly different level.
Linear regression of in tissue response times for lactate and glucose sensors yielded $r=0.03$ ($n=6$, $p<0.05$, $\alpha=0.03$). The linear regression between subcutaneous levels of lactate and glucose was $r=0.59$ ($n=6$, $p<0.05$, $\alpha=0.21$) at $t=0$ minutes and $r=0.81$ ($n=6$, $p<0.05$, $\alpha=0.50$) at $t=45$ minutes.

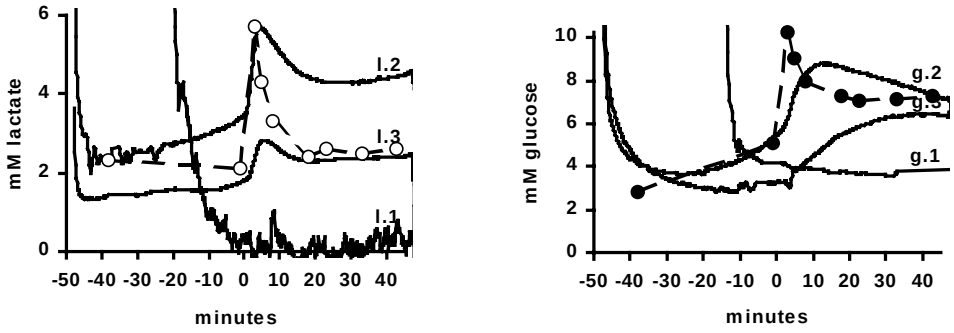

Figure 3
Lactate (left) and glucose levels (right) after an intravenous lactate and glucose bolus at t = 0 minutes. Measurements with subcutaneous sensors (~) and in venous blood samples (o lactate, and • glucose) Shown are the fastest (g.1, l.1) and slowest (g.2, l.2) responding sensors in connective tissue, and a typical sensor response in adipose tissue (g.3, l.3). The curve at the start of each graph reflects the settling of the sensor after insertion in tissue.

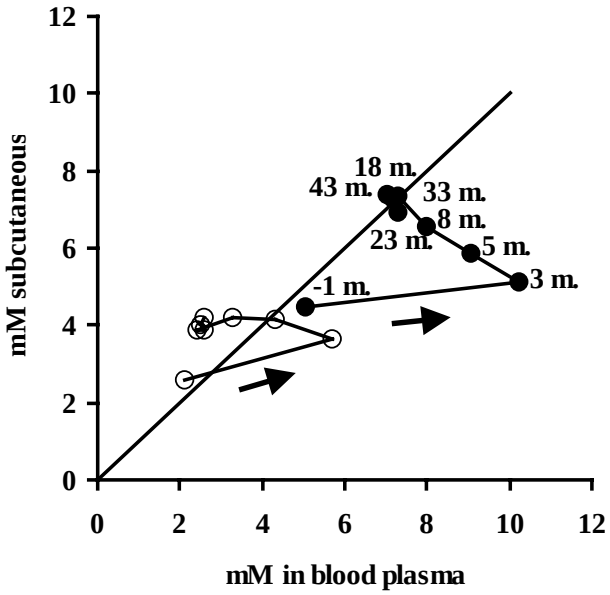

Figure 4
Lactate (o) and glucose (•) levels in venous blood versus subcutaneous connective tissue (average of six sensors). Subsequent measurements (➨) are indicated with the time in minutes (-1m. to 43 m.) after an intravenous lactate and glucose bolus at t = 0 minutes.

Conclusions

The results of this in vivo exploration of placements of a thin-film sensor in different superficial tissues indicate some promising directions for further clinical research. Adipose and striated muscle tissue placed sensors showed either zero currents or highly variable baseline levels, both without any response to intravenous lactate-glucose injections. These sensors appear kinetically separated from the intravascular compartment as they show no response. The near zero electric currents indicate that these sensors are even barely in contact with the interstitial electrolytes.

In subcutaneous loose connective tissue, sensors showed baselines close to intravascular levels and showed less variability (standard deviations lower). The proper placement of each individual sensor close to the intravascular compartment was confirmed quickly and easily with an intravenous injection, as the speed of distribution from the intravascular to the tissue compartment is reflected in the in vivo response time of each sensor. The positive and negative results of simultaneous glucose and lactate measurements parallel for different placements. Concerning a possible lactate and glucose interdependency, the subcutaneous response times showed no correlation. The t0 and t45 minutes subcutaneous glucose and lactate levels do suggest a positive correlation, although with a low power. An underlying process may explain these observations. A possible explanation is a poorer peripheral tissue perfusion due to fluid loss during the experiment. This can cause the intravascular glucose level to remain high after the bolus injection, and stimulate glucose to lactate metabolism in tissues. Whatever may be the explanation, the difference in blood and subcutis kinetics appears small for glucose, but significant for lactate.

The presented results are to our knowledge the first of a continuous lactate sensor directly in vivo. Despite the need for better monitoring of patients in critical care, few lactate sensors have been proposed so far. Off-line microdialysis experiments have been done in patients[6;15]. Further, continuous needle-type, thick-film, and thin-film sensors have been applied successfully to (on-line) ex-vivo blood and microperfusion samples[4;5;13;16]. The direct subcutaneous monitoring proposed in this study will be less of a patient burden than techniques using continuous blood sampling or heparin.

The common placement of sensors in adipose tissue is questioned by our results comparing sensor characteristics in different tissues. Discouraging results with lactate and glucose monitoring probes in the past may be due to improper placement and possibly local glucose to lactate metabolism. A quick check on

proper placement appears feasible with an intravenous injection, as demonstrated here with an extra cross check of glucose and lactate sensors for each other. This method may be useful for improving reliability of sensors, since current (subcutaneous glucose) sensors need multiple blood samples and much patience to calibrate, but these methods still gives only information on the general correlation of measurements in large groups of patients. With the here presented method, the proper placement and timeliness can be checked in principle of each single sensor placed in each individual patient. The future application of sensors may be enhanced through thus improving the reliability.

Future research can benefit from the results of this exploration. Recently, we compared microdialysis probe placement in connective tissue with adipose tissue in human volunteers, obtaining essentially the same results as in this exploration[17]. So, subcutaneous loose connective tissue appears to be the tissue of choice for sensor placement, rather than the now generally utilised periumbilical adipose tissue. The observed delays in connective tissue may be corrected in future by calculation with parameters which can be derived from the response curve to the intravenous injection (mathematical deconvolution of sensor response[18]). Whether the thin-film sensor will prove to be timely for clinical decision making, appears promising, but has to be a subject of subsequent clinical investigations. A possible interdependence of tissue glucose and lactate has to be investigated also, because these measurements may improve one other by accounting for metabolism. A possible dependency of measurements on tissue perfusion may offer additional information of interest for monitoring critically ill patients.

The present exploration has focused on the influence of anatomic placement of monitoring devices which has been paid as yet little attention. A limited number of observations has been done, so any conclusions should be drawn cautiously. Implantation procedures appear relevant for sensor functioning in vivo. Loose connective tissue was shown to be the preferential place for sensing in pigs. Future investigations for the improvement of subcutaneous sensing in patients should consider the influence of placement on monitoring.

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